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Progesterone Receptor Function from a Behavioral Perspective

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Hormonal induction of sexual receptivity in ovariectomized female mice can be effectively reinstated by sequential administration of estradiol and progesterone. In this regard, mice appear to be similar to other rodents. While it is generally accepted that hypothalamic progesterone receptors function as estradiol-induced transcription factors in the induction of sexual receptivity in rats, hamsters, and guinea pigs, relatively little is known about their role in the mouse, a species which exhibits genotypic and strain differences in the responsiveness to steroid hormones. Using a transgenic mouse carrying a null mutation for the progesterone receptor by gene targeting, we examined the role of the progesterone receptor as a coordinator of key regulatory events in the induction of sexual receptivity. A concordance between hypothalamic progesterone receptor levels and behavioral responsiveness was established by comparing the homozygous mutant, heterozygous mutant, and wild-type littermates. The behavioral and biochemical findings reveal the importance of estradiol-induced progesterone receptors for the expression of sexual behavior in female mice. The behavioral response of the two parental mouse strains from which the recombinant genotype was generated was also examined. As an extension of our earlier studies on the ligand-independent activation of progesterone receptors by neurotransmitters, the behavioral effect of dopamine in the facilitation of sexual receptivity in mice was also examined. The studies provide further evidence that steroid hormone receptors function as general transcription factors to achieve the integration of neural information in the central nervous system, and they assign a more important role for progesterone receptors than hitherto envisioned.

The ovarian steroid hormones estradiol (E_2) and progesterone (P) have profound modulatory influences upon the central nervous system resulting in changes in reproductive physiology and behavior (Blaustein and Olster, 1989; Pfaff *et al.*, 1994). In gonadally intact female rodents, the sequential release of ovarian estradiol and progesterone integrates the appearance of sexual behavior (heat, behavioral estrus) with ovulation (Beach, 1942; Blaustein and Olster, 1989 and references therein). This behavior can be abolished by ovariectomy and restored by timed exogenous treatment with both E_2 and P or by high doses of E_2 alone. Sequential treatment with E_2 and P maximizes the probability that the female will assume the lordosis posture, a primary behavioral component of female sexual behavior, when mounted by a conspecific male. The sequential hormonal regimen also allows lower doses of each of the hormones to result in a more predictable onset and termination of the period of sexual behavior and lordosis duration. Since the occurrence of female sexual behavior can be manipulated in a predictable fashion by E_2 and P and can be measured with a high degree of validity and reliability, this behavioral model has been extensively used for investigating the mechanism of hormone action in the brain.

One means by which hormones exert their physiological effects is via their intracellular receptors functioning as ligand-dependent nuclear transcription factors, regulating the expression of genes and genomic networks (Romano *et al.*, 1989). With the advent of cloning and molecular techniques, novel molecular biological approaches are being used to elucidate the cellular and molecular mechanisms underlying hormone–receptor interactions in the central nervous system. In this paper we will provide a synopsis of studies on the role of the progestin receptor (PR) in the facilitation of sexual behavior in female rats, guinea pigs, and mice. We will review the neurotransmitter and PR interactions that are thought to be involved in the modulation of these behaviors. We will present an overview of our observa-

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tions on the role of PR in steroid hormone- and neurotransmitter-facilitated sexual behavior using transgenic mice carrying a null mutation for the PR gene. The studies reinforce our earlier observations on the role of PR as being not only a ligand-dependent transcription factor but also a coordinator of the pathway by which the neurotransmitter dopamine alters steroid-receptor-dependent sexual behavior in female rodents. The advantages as well as the caveats in the interpretation of the behavior when using null mutants will also be discussed.

MECHANISM OF ACTION OF PROGESTERONE

Steroid hormones, including progestins, are believed to exert their physiological effects primarily by binding to cognate intracellular receptors (Jensen *et al.,* 1968; O'Malley, 1990). PRs are ligand-(hormone) inducible members of a superfamily of transcription factors that undergo conformational changes upon binding to the hormone, leading to their nuclear translocation, dimerization, and DNA binding (Beato *et al.,* 1995). The ligand-induced receptor conformation is capable of recruiting general transcription factors (GTFs) to the promoter and altering chromatin structure, leading to the formation of the preinitiation complex and the modulation of target gene expression (Klein-Hitpass *et al.,* 1990). Receptor phosphorylation occurs following ligand activation of PR and is thought to contribute to the receptor's transactivation potential (Denner *et al.,* 1990; Bagchi *et al.,* 1992). Both cytoplasmic and nuclear phosphorylations occur after the hormone binds to the cognate intracellular receptor, followed by a final round of phosphorylation after receptors bind to the DNA prior to target gene activation (Takimoto *et al.,* 1992).

As in the peripheral reproductive tissues, the fundamental effects of P on sexual behavior are believed to be mediated by the interaction of hormone–receptor complexes with the genome (Blaustein and Olster, 1989). Spatial, temporal, and functional correlations suggest that E_2-induced PR, upon occupation by its cognate ligand, functions as a transcriptional mediator, regulates transcription of target genes, and affects the neural networks involved in the control of sexual behavior (Pfaff *et al.,* 1994). The time course of activation and termination of sexual behavior parallels the estradiol benzoate (EB)-induced increase and decline in progestin binding sites in the hypothalamus and preoptic area of the brain (Blaustein and Feder, 1979). The increase in the concentration of unoccupied, hypothalamic cytosol PRs, followed by the accumulation of occupied, nuclear PRs in response to a behaviorally effective dose of P correlates well with the onset of sexual behavior in rodents, indicating that the hormone facilitation of sexual behavior is a two-step intracellular receptor-mediated mechanism (Blaustein and Feder, 1980). Furthermore, the inhibition of P-facilitated sexual behavior by the P antagonist RU 486 demonstrates that the hormone's effects are mediated by PRs (Blaustein and Olster, 1992).

Additional support for an integral role of PRs in P-facilitated sexual behavior comes from studies of P-induced refractory period to further facilitation by P in guinea pigs. During the refractory period, when the animals are hyposensitive to P, the concentration of unoccupied hypothalamic PRs is decreased. Treatment with P results in low levels of occupied nuclear PRs, suggesting that the hyposensitivity (and the resulting termination of the period of sexual behavior) is due to the failure to accumulate an adequate concentration of occupied PRs in response to P (Blaustein and Olster, 1989; Blaustein and Feder, 1980).

Intrahypothalamic application of inhibitors to RNA and protein synthesis blocks E_2 and P-facilitated lordosis also suggesting the involvement of genomic mechanism in the control of female reproductive behavior (Whalen *et al.,* 1974; Rainbow *et al.,* 1982). The importance of PRs in the regulation of P-facilitated sexual behavior comes from recent work in which antisense oligonucleotides to PR mRNA have been administered into the brain. We and others have provided additional evidence for the involvement of intracellular PR in P-facilitated sexual behavior, by infusing the antisense oligonucleotides to PR into the cerebral ventricles (Mani *et al.,* 1994a) or ventromedial hypothalamus (Pollio *et al.,* 1993; Ogawa *et al.,* 1994). The results of all these studies support the notion that facilitation of sexual behavior by P in rodents is mediated predominantly via E_2-induced genomic activation of neural PR.

While most of the data concerning P regulation of sexual behavior are consistent with the genomic mechanism, other steroid hormone effects have been reported that are not compatible with a slower genomic mechanism. These studies indicate the existence of ''nongenomic'' effects involving putative cell surface receptors in the regulation of female reproductive behavior by P. P is believed to exert these nongenomic effects by binding to membrane receptors (Ramirez *et al.,* 1996) that gate ion channels (Gee *et al.,* 1987). A rapid mechanism of action has been observed for both E_2 + P in the induction of release of LHRH (Ramirez *et al.,* 1990), release of dopamine and acetylcholine (Meiri, 1986), release of excitatory amino acids (Smith *et al.,* 1987), changes in neural activity (Kelly *et al.,* 1977, Havens and Rose,

1988), and norepinephrine-stimulated cAMP synthesis (Etgen *et al.*, 1992). Interaction between membrane-bound and intracellular PRs has been observed in the facilitation of sexual behavior in female hamsters (DeBold and Frye, 1994), suggesting that both genomic and nongenomic mechanisms probably act in concert rather than independently to facilitate sexual behavior. The mechanisms of such an interaction remain to be elucidated.

STEROID HORMONE–NEUROTRANSMITTER INTERACTIONS IN SEXUAL BEHAVIOR

The involvement of neurotransmitters and neuropeptides in cellular processes by which steroid hormones influence sexual behavior in female rodents is well known. While acetylcholine, norepinephrine, serotonin (acting via 5-HT_2 receptor subtype), dopamine (acting via receptor subtype D_1), and the neuropeptides LHRH, TRH, prolactin, oxytocin, substance P, and $GABA_A$ are considered facilitatory, serotonin (acting via receptor subtype 5-HT_{1A}), dopamine (acting through receptor subtype D_2), opioids, CRF, α-MSH, ACTH, β-endorphin, neuropeptide Y, cholecystokinin, and glutamate are considered inhibitory on sexual behavior (Pfaff *et al.*, 1994). While it is evident that complex interactions do exist among neurotransmitters, neuropeptides, and steroid hormones in bringing about the facilitation or inhibition of female reproductive behavior, the cellular and molecular pathways of these interactions are not completely understood.

Steroid hormones have been shown to influence sexual behaviors by alteration in neurotransmitter biosynthesis and release (Nock and Feder, 1981), allosteric modulation of membrane receptors (Rainbow *et al.*, 1980), changes in neurotransmitter receptor densities, and interactions with G-protein coupling and subsequent intracellular signaling pathways (Etgen *et al.*, 1992). This steroid hormone–neurotransmitter interaction, however, is not unidirectional. Not only do steroids affect neurotransmission, but changes in neurotransmission can also alter steroid activity. In the past decade, a wide array of techniques have been used to investigate the influence of neurotransmitters on steroid receptor modulation and a wide variety of studies exist in the literature (Blaustein, 1992). A discussion of all the findings on various neurotransmitters and their effects on hormonal regulation is beyond the scope of this article. We will restrict our focus to the regulation of PR by the neurotransmitter dopamine.

Pharmacological studies with dopamine agonists and antagonists have implicated a role for this neurotransmitter in the regulation of sexual behavior in female rodents (Foreman and Moss, 1979; Ahlenius, 1993). *In vivo* brain microdialysis studies in female rats have demonstrated an increased release of dopamine from the VMH, the steroid receptor-rich region of the hypothalamus known to be involved in hormonal regulation of sexual behavior, at the time of mating (Vathy and Etgen, 1989). Autoradiographic studies in the rat brain suggest the presence of E_2-concentrating neurons in the hypothalamus and preoptic areas that also have afferent input from catecholaminergic neurons (Heritage *et al.*, 1980). Similarly, estrogen receptor (ER) immunoreactive neurons of the female guinea pigs are believed to be innervated by dopamine-β-hydroxylase immunoreactive neurons in the hypothalamus (Blaustein, 1992 and references therein). Tyrosine hydroxylase immunoreactive neurons have been shown to be in close association with and synapsing upon PR-containing neurons of the hypothalamus in the guinea pig (Blaustein and Turcotte, 1989), the rat (Horvath *et al.*, 1992), and the monkey (Herbison *et al.*, 1995).

The role of the neurotransmitters dopamine and norepinephrine in the regulation of ER and PR concentrations in the hypothalamus and pituitary gland in rat and guinea pig brain is well known (Nock *et al.*, 1981). In rats, inhibition of dopamine-β-hydroxylase, the enzyme that catalyzes the conversion of dopamine to norepinephrine, results in a transient increase in hypothalamic cytosol estrogen receptors followed by a pronounced decrease (Blaustein, 1992). It has been suggested that the early increase in receptor levels is thought to be due to an increase in dopamine levels, as similar increases have been reported under some conditions with dopamine agonists (Gietzen *et al.*, 1983).

A decrease in the concentration of E_2-induced cytosolic PRs in the guinea pig hypothalamus was observed upon administration of a dopamine-β-hydroxylase inhibitor or antagonist of α_1-adrenergic receptors (Nock *et al.*, 1981; Thornton *et al.*, 1986). This decrease in cytosolic PRs after dopamine-β-hydroxylase inhibitor was accompanied by an increase in nuclear PRs (Blaustein, 1985). These studies suggest that alterations in catecholaminergic activity may cause changes in hypothlamic PRs, similar to the steroid hormone actions in peripheral reproductive tissues (Jensen *et al.*, 1968; Tsai and O'Malley, 1994). While these studies suggest that neurotransmitters regulate the concentration of steroid receptors, the studies discussed in the following section support the notion that this also involves a genomic mechanism.

DOPAMINE–PROGESTERONE RECEPTOR INTERACTION: A LIGAND-INDEPENDENT MECHANISM

Although the conventional model of steroid receptor activation assumes that a cognate ligand (hormone) is absolutely required for the activation of the receptor (ligand-dependent), several studies in the past few years have shown that in some circumstances, some classes of steroid receptors can be activated by factors other than their cognate ligands. The earliest observation that P-dependent, PR-mediated transcription could be activated in a ligand-independent manner by cAMP (8-bromo-cAMP) in the absence of P (Denner *et al.*, 1990) was followed by studies demonstrating that the avian PR could be activated by other pathways as well. Power *et al.* (1991a) showed that dopamine could activate the chicken ovalbumin upstream promoter transcription factor, a member of steroid receptor superfamily. This ligand-independent activation by dopamine and dopamine agonists, which resulted in the translocation of the receptor from the cytoplasm to the nucleus, was also demonstrated in an *in vitro* cell transfection system with PRs as well (Power *et al.*, 1991b). The specificity of the dopamine agonists suggested that a receptor of D_1 subtype is responsible for this activation. Recent studies on growth factor-mediated activation of PRs (Zhang *et al.*, 1994) and estrogen receptors (Aronica and Katzenellenbogen, 1993) and LHRH activation of PRs (Turgeon and Waring, 1994) also suggest that these effects probably are mediated via membrane-associated signaling cascades rather than the factors acting directly as ligands for PR.

Antisense oligonucleotides have been used successfully to reduce the levels of EB-induced PRs and inhibit P-facilitated lordosis in female rats (Mani *et al.*, 1994a; Pollio *et al.*, 1993; Ogawa *et al.*, 1994). Using antisense oligonucleotides to PR mRNA, we reported that dopamine facilitates the expression of sexual behavior by a process involving ligand-independent activation of the PR. Intracerebroventricular (icv) administration of a dopamine receptor stimulant apomorphine or the D_1 agonist facilitated sexual behavior in female rats mimicking the effects of P (Mani *et al.*, 1994b). The facilitatory effect of dopamine was specific to the D_1 receptor subtype, confirming and extending the earlier report in which dopamine agonists were infused into the hypothalamus and preoptic area (Foreman and Moss, 1979). While the facilitatory effect of a dopamine agonist could be blocked by icv administration of a D_1 receptor antagonist, it could be inhibited by of PR antagonists or antisense oligonucleotides to PR mRNA (Mani *et al.*, 1994b). Thus, facilitation of sexual behavior by dopamine requires the presence of intact, intracellular PR. Based on these observations we suggested the existence of cross-talk between membrane receptors for dopamine and intracellular PR. We further postulated that this dual mechanism for activation of steroid receptors may be an important communication mechanism by which neurotransmitters affect steroid receptor-dependent gene expression and behavior in female rodents. The precise mechanism by which dopamine activates progestin receptors is yet unknown, although it has been suggested to involve the phosphorylation of the receptor or some specific coactivator or other messenger systems associated with the receptors (McInerney *et al.*, 1996; Smith *et al.*, 1996).

ADVANTAGES OF USING PROGESTERONE RECEPTOR KNOCKOUT MICE (PRKO) TO STUDY SEXUAL BEHAVIOR

PR exists in two different molecular forms, termed PR-A (79–94 kDa) and PR-B (101–120 kDa), in various species. These two isoforms, different only at the amino terminus, are derived from transcripts initiated from two distinct estrogen-inducible promoters within a single PR gene (Conneely *et al.*, 1987; Kastner *et al.*, 1990). The ratio of the two isoforms has been shown to differ among species, being equimolar in the human and avian PR (Weigel *et al.*, 1995; Horwitz and Alexander, 1983) and predominantly of the smaller A form in rodents (Ilenchuk and Walters, 1987). The distribution and functional role of these isoforms in the brain have not been defined, especially in the context of sexual behavior. It is possible that the isoforms have distinct opposing biological roles as have been described in cell culture studies and have a bearing on behavioral responses. Pharmacological approaches using ligand antagonists and agonists cannot distinguish between the molecular isoforms A and B.

While antisense oligonucleotide technology offers several interesting possibilities to manipulate the different isoforms to various genes specifically, the occasional nonspecific or toxic actions and/or incomplete effects have been considered distinct disadvantages. To alleviate these issues and define the responses that are specifically mediated by intracellular PR in sexual behavior, we turned to a genetically altered mice strain carrying a null mutation for the PR gene (Lydon *et al.*, 1995).

Since the disruption of the mouse PR gene is targeted

toward the intracellular PR, this model could be advantageous in studying the nongenomic effects without the interference of genomic effects. This is based upon an assumption that the recently cloned P membrane-binding protein from the vascular smooth muscle of porcine liver is similar to that in the brain. Its molecular weight and its genomic sequence (Falkenstein et al, 1996) indicate no similarities to the intracellular PR, suggesting that the proteins are products of two different genes.

The details pertaining to the generation of the PRKO mouse and the defects in reproductive tissues have been described in great detail elsewhere (Lydon *et al.*, 1995). Briefly, the phenotype of animals includes uterine hyperplasia and limited mammary gland development. The female homozygotes (PRKOs) are sterile and their male siblings are fertile, suggesting that the involvement of PR in male reproduction is minimal. No estrous cyclicity was observed based on vaginal smears, and the PRKOs were unable to ovulate, suggesting that the hypothalamo-pituitary–ovarian axis was affected.

HORMONAL INDUCTION OF SEXUAL RECEPTIVITY IN PRKO MICE

A summary of our recent work using PRKOs in the hormonal induction of sexual receptivity (Mani *et al.*, 1996) is discussed below.

Effects of Hormonal Priming and Testing Experience

Hormonal induction of sexual receptivity in ovariectomized mice, as in other rodents, can be reinstated effectively by sequential administration of EB and P (Ring, 1944; McGill, 1961). Large strain and genotypic differences in the hormonal requirements for the induction of receptivity are known to exist in female mice (McGill, 1961; Gorzalka and Whalen, 1976). Since two different mouse strains, C57BL/6 and 129SvEv, were used to generate PRKOs, we performed a baseline study on the hormone inducibility of sexual behavior in the parental strains in response to mating by male mice of the same strains. Estradiol benzoate (EB; 0.5 μg) alone was not capable of inducing the lordosis response in response to mounting by the male in either strains at any of the doses examined. This suggests that under our testing conditions, P is essential for the induction of sexual receptivity in female mice of these strains. In addition, both strains of mice appeared to require at least 4 weeks of repeated weekly hormonal priming and testing experience for the induction of maximal sexual receptivity. Under the same experimental paradigm, PRKOs did not exhibit any lordosis response, while their wild-type (Wild) and heterozygous (Hetero) littermates showed responses similar to those of the parental strains (Fig. 1). While this requirement differs from female members of a variety of rodent species, it is consistent with earlier work in mice (McGill, 1961; Gorzalka and Whalen, 1976, Edwards, 1970).

Hypothalamic Progesterone Receptors and Lordosis

We analyzed the hypothalamic cytosol PRs in PRKO mice and compared them to those from age-matched Wild and Hetero female mice. *In vitro* one-point binding analyses of EB-induced cellular PRs in the mediobasal hypothalamus demonstrated a 70% reduction in estradiol-induced binding in the PRKO females, while heterozygous females had a 40% decrease in EB-induced PRs compared to the wild types (Fig. 2A). Unfortunately the nonavailability of sufficient tissue for performing scatchard analysis led us to use the one-point binding assay for progestin binding. It is not possible to determine if the binding in the PRKO represents a residual low level of high-affinity binding (i. e., PR) or the low-affinity binder known to bind ^{3}H-labeled R5020. Nevertheless, it is clear that the PRKOs have at least a greatly reduced level of progestin binding.

We next correlated PR levels with the sexual receptivity in the PRKO female mice. Ovariectomized PRKO mice and their heterozygous and wild-type littermates were injected with EB and P and tested for sexual behavior in response to mating by wild-type male mice. Both wild-type and heterozygous mice exhibited high levels of lordosis, while PRKO females showed a minimal response. EB alone did not induce lordosis in any of the mice tested at the dose used. As with the progenitor strains, repeated EB and P priming and mating experience over a period of 3–4 weeks resulted in high levels of lordosis in wild-type and heterozygous mice. Similar treatments, however, had no effect on the lordosis response of PRKO mice, as demonstrated by the display of minimal responsiveness by these females during any of the tests (Fig. 2B).

Estradiol-Induced Sexual Behavior

The PRKO mice were also tested for their ability to respond to daily injections of EB at high doses (10 μg) for up to 10 days. Testing for receptive behavior to mounting by a male was done on Days 1–4 after EB injection and on every alternate day subsequently. Such

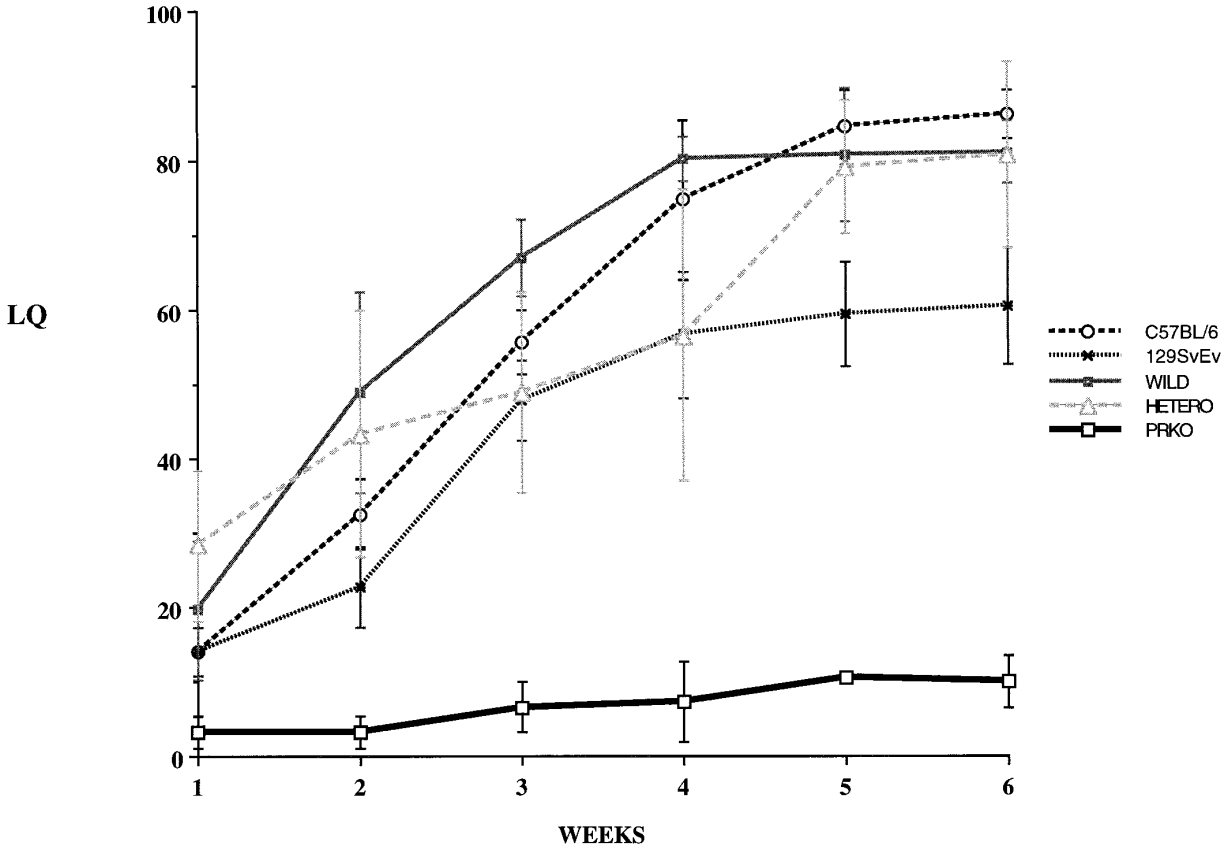

FIG. 1. Estradiol benzoate (EB)-progesterone (P) induction of sexual receptivity in ovariectomized C57BL/6, 129SvEv, PRKO, and their wild-type and heterozygous littermates. Ovariectomized female mice were subcutaneously administered EB (0.5 μg), followed by P 48 hr later. The hormones were administered every week for 6 weeks and the animals were tested weekly for sexual receptivity to mounting by wild-type male mice (F_2 generation littermates) 6 hr after P administration. Values presented are means LQ ± SEM (n = 6–8 animals for each group).

a paradigm facilitated sexual receptivity in the wild types, although the lordosis quotients were not as high as those obtained with EB + P treatments. Surprisingly, EB-induced lordosis response was also observed in PRKOs, demonstrating that their ability to respond and exhibit lordosis is not compromised (Fig. 3). These observations indicate that PRs are not required for the EB-induced sexual behavior, confirming the earlier observations in rats (Blaustein *et al.*, 1987).

Progesterone Receptors Are Involved in Dopamine Facilitation of Sexual Behavior

In earlier studies using antisense oligonucleotides to PR in rats, we demonstrated the necessity of intracellular PRs in mediating the effects of dopamine in the facilitation of sexual behavior in female rats (Mani *et al.*, 1994b). In order to test this hypothesis further, we examined the facilitation of sexual behavior by the D_1 dopamine agonist, SKF 38393, in PRKO mice. A time and dose response study was initially performed after icv administration of the dopamine agonist in the wild-type littermates. Icv administration of SKF 38393 facilitated high levels of lordosis in wild-type littermates having a full complement of PRs, while the PRKO females having reduced cytosol PRs showed low levels of lordosis (Fig. 4). While these studies provide further evidence that PRs are required for at least one behavioral effect of dopamine in female rodents, its role in other behavioral components facilitated by dopamine needs further evaluation.

CAVEATS IN THE INTERPRETATION OF BEHAVIOR USING NULL MUTANT MICE

The genetically altered mouse model provides an attractive system for molecular dissection of the complex relationships between steroid receptors and other experiential, social, and environmental influences that affect

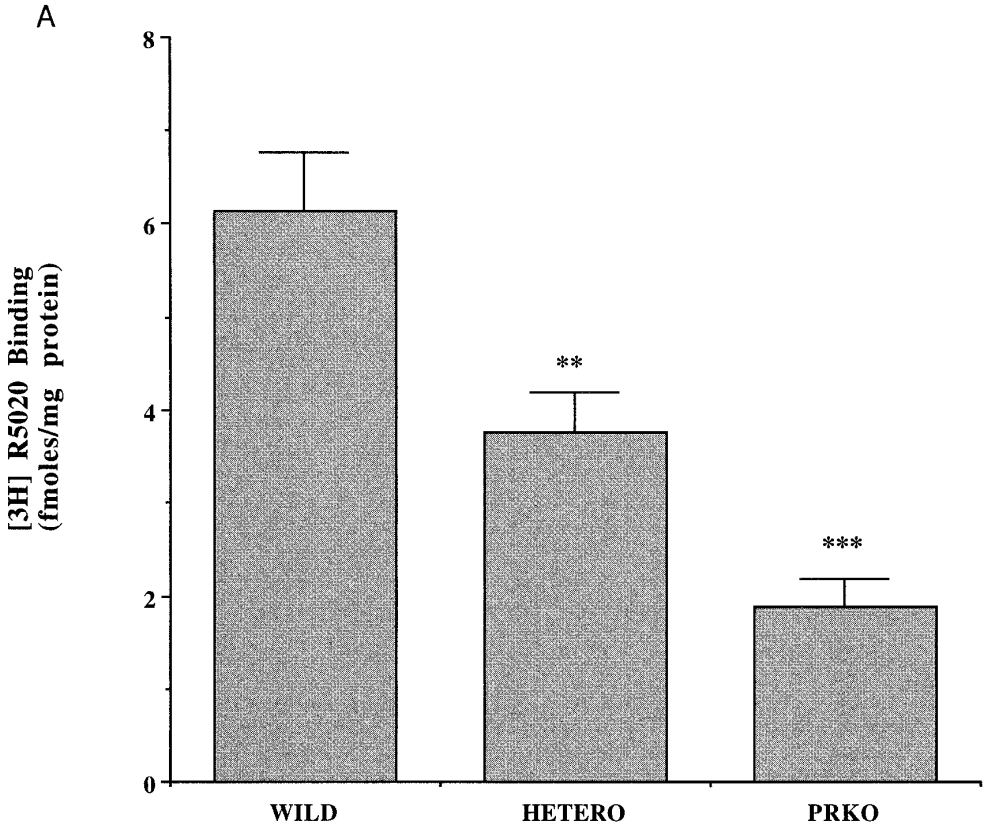

FIG. 2A. Progestin binding in the hypothalamus of PR mutants. Mean (± SEM) PR concentration in cytosol from the hypothalamus of ovariectomized mice, injected sc with 0.5 μg of EB. The animals were killed 48 hr after EB administration, the hypothalamus and cortex were dissected and homogenized, and progestin-binding assays were performed on the cytosol. Cytosol from cortex was used as control. Each point is the mean of six independent determinations. **Significantly different from wild-type receiving identical hormone treatments ($P < 0.01$). ***Significantly different from wild-type receiving EB + P treatment ($P < 0.001$).

neural response and behavior. While behavioral observations in null-mutant mice are subject to several caveats as have been discussed and/or demonstrated by several others in this issue (e.g., Crawley and Paylor, 1997; Nelson, 1997; Young *et al.*, 1997; Rissman *et al.*, 1997), and several comprehensive commentaries are available (Gerlai, 1996; Crawley, 1996), a synopsis of the caveats is discussed below.

In order to understand several of the caveats, we have to consider the genetics involved in generation of a null mutant mouse. In most of the studies, the gene targeting is carried out in embryonic stem cells derived from the mouse strain 129SvEv and introduced into a blastocyst stage embryo. The chimeric embryos are allowed to develop to term in foster mothers, raised to adulthood, and then mated to wild-type mice from a different strain, for example, C57BL/6. The offspring from the F_1 generation from such matings are heterozygous for the mutant allele and have one set of chromosomes from strain 129 SvEv and another from strain C57BL/6. When these heterozygous mice are mated with their siblings they will produce the F_2 generation in which homozygous null mutant, heterozygous mutant and wild-type mice are found segregated according to Mendel's Law.

(1) As null mutant mice are often hybrids of two mouse strains, they have chromosomes from both strains. Since strain variations are known to exist in the display of sexual behavior in mice, caution must be exercised in the interpretation of behavior when using mutant mice. For this reason, we have included the parental strains as controls in addition to their wild-type and heterozygous littermates. This enables us to determine if the behavioral patterns were drastically different from the parental strains.

(2) Compensatory changes, if any occur, will depend upon the targeted gene as well as on the background genotype. That is to say, targeted disruption of one gene may lead to a differential expression of another

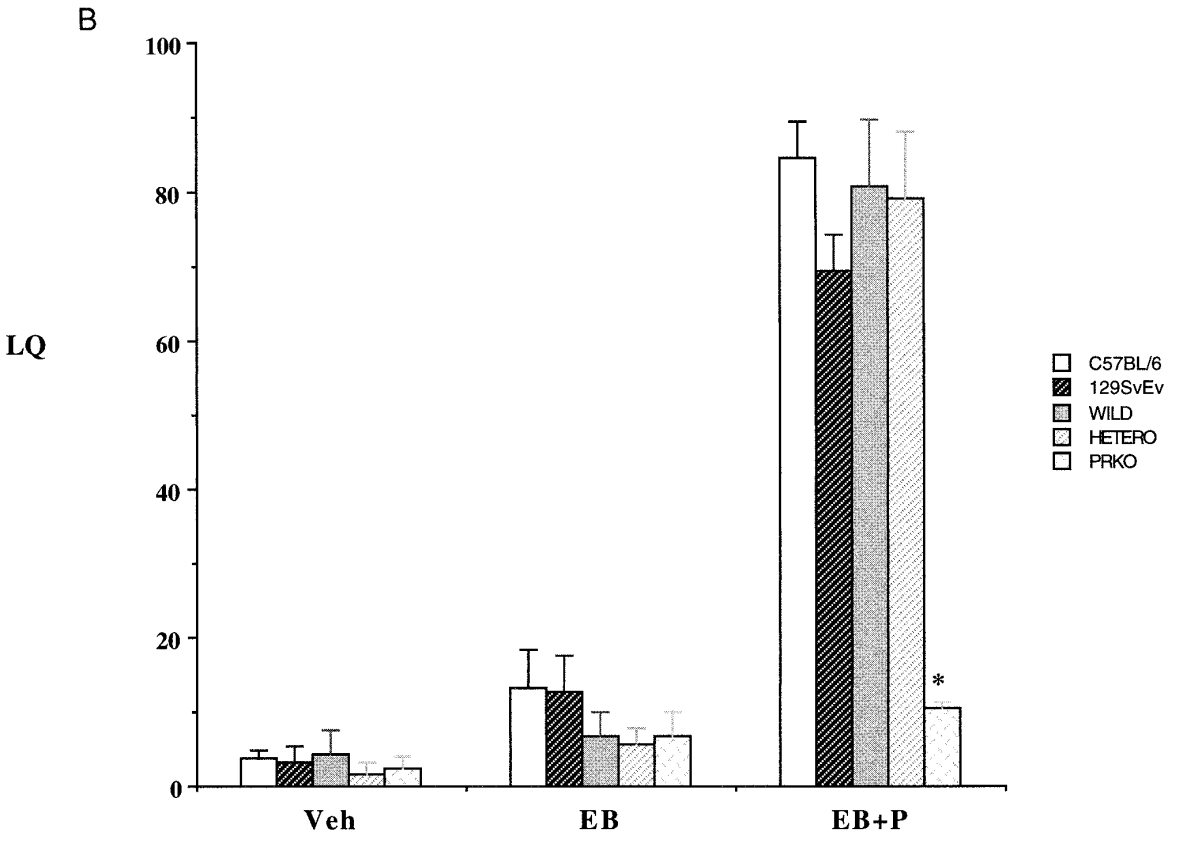

FIG. 2B. EB + P induction of sexual receptivity in PR mutants. Ovariectomized mice from the three genotypes and parental strains were administered 0.5 μg EB, followed by P (100 μg) 48 hr later. The hormones were administered every week for 6 weeks and tested weekly for sexual receptivity. Values are presented as mean LQ ± SEM from Week 4 of testing. Statistically significant ($^*P < 0.05$) differences were observed in EB + P treated PRKOs as compared to wild-type animals that received the same treatments. ($n = 6$ for all groups). Values are presented as mean LQ ± SEM.

gene(s). Similar regulatory changes in the genetic background may lead to different phenotypic effects in the null mutant, making the phenotypical changes difficult to interpret.

(3) The recombination pattern is different even among littermates. Since the F_2 population constitutes mice with recombinant genotypes derived from the two parental mouse strains, the recombination pattern (i.e., which locus contains alleles from 129 and which contains alleles from C57 and whether these are in the homozygous or heterozygous form) might be different among littermates. Thus, the alleles of wild-type littermates might be different from those of the mutants, not only at the locus of the gene of interest but also at other loci. This problem is generally alleviated by assessing a larger number of animals and decreasing the possibility of sampling error associated with recombination pattern differences among littermates.

(4) The observed effects could be due to the null mutation itself or to the background genes linked to the target locus. As the targeted mutation is carried out in 129 SvEv embryonic stem cells, the alleles of genes surrounding the targeted locus will carry genes of 129SvEv-type in the null mutant mice. The nonmutant control animal will have C57BL/6 type alleles. Thus, the phenotypical differences might be due either to the introduced null mutation or to the background genes linked to the targeted locus.

(5) Since the mutation exists from the early stages of embryogenesis throughout the stages of development, any or all of the compensatory processes during the development will not be reflected in the behavioral studies when using knockout mice in adulthood. The role of PRs as we perceive using PRKOs is based on our observations in an animal that has developed to adulthood in the complete absence of PR and does not take into account the developmental effects dependent upon PR and the alternative pathways and genetic redundancies that could exist. At the current time there is no logical way to address this issue in the current

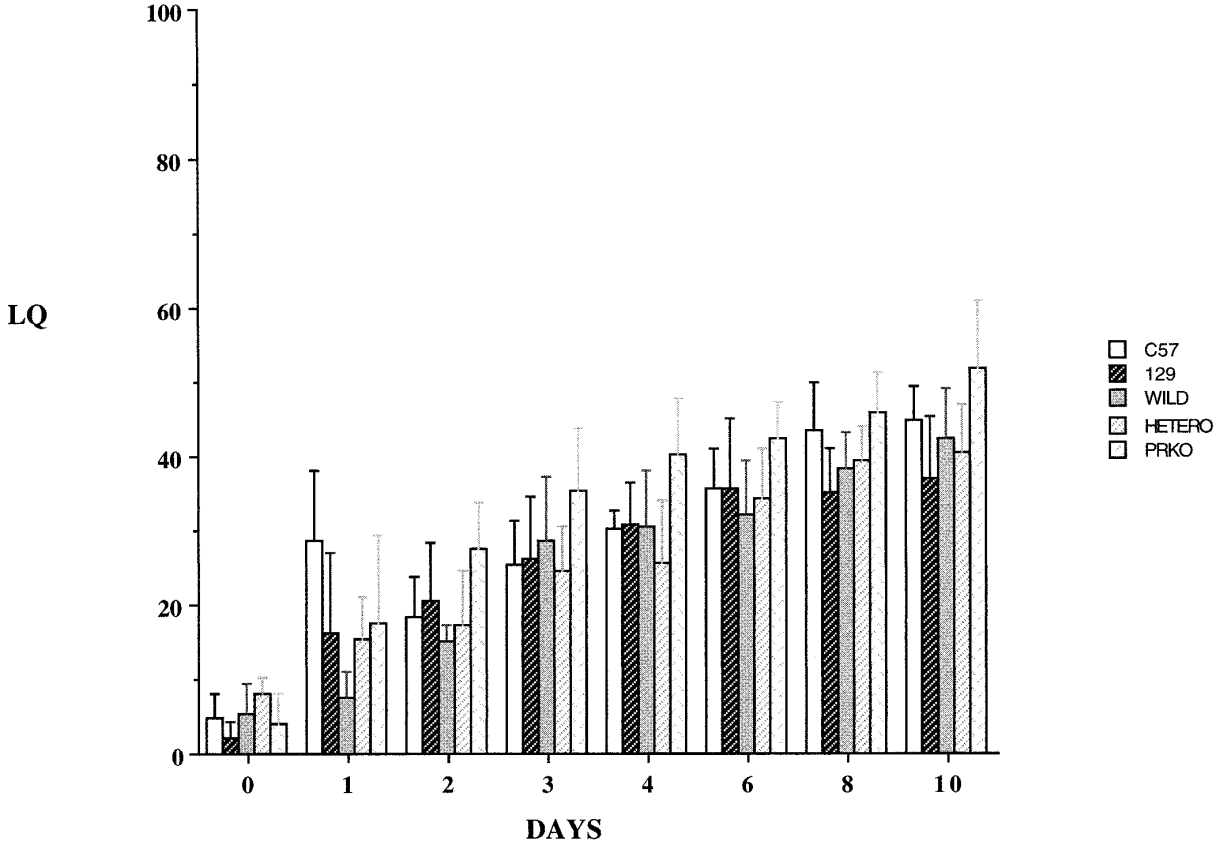

FIG. 3. Estradiol benzoate-induced sexual behavior in PR mutants. Ovariectomized animals were administered daily injections of EB (10 μg) for 10 days. They were tested for lordosis response to mounting by a male on Days 1–4 after EB injection and on alternate days subsequently. Values are mean LQ ± SEM (n = 8–10 animals in each group).

model given that the role of PR in development is still an enigma. A potential solution would be to generate inducible knockouts, which would allow the investigator to switch the gene of interest on or off at particular times and enable the comparision of the pre- and post-induction phenotypes.

While many of these caveats apply to all the analyses of physiological processes when using animals generated by the targeted gene knockout technology, these are inherent problems for which there are no immediate solutions. Providing adequate controls for as many variables as possible may reduce but not alleviate some of these problems as discussed above. Nevertheless knockout technology has certainly provided a means to dissect the molecular mechanisms underlying complex behavioral interactions.

SUMMARY AND CONCLUSIONS

The data reviewed above clearly support the idea of a unique and critical role for PRs in the regulation of sexual behavior. By acting as a point of convergence for dopamine- and P-initiated signaling pathways, it functions as a critical coordinator of key regulatory events associated with reproductive behavior. In addition, behaviorally relevant stimuli have also been shown to activate PRs in a ligand-independent manner, demonstrating a role for PRs in sexual behavior induced by social interaction (Auger *et al.*, 1997). The generally accepted idea that PR functions strictly as a ligand-dependent transcription factor to initiate gene transcription and subsequent behavioral effects is undergoing modifications to include the ligand-independent activation pathways initiated by neurotransmitters and growth factors (Ignar-Trowbridge *et al.*, 1993; Aronica and Katzenellenbogen, 1993). While the precise mechanism for this activation is not understood, it is possible that regulation of phosphorylation dynamics of the steroid receptors is altered by signal transduction cascades initiated from cell membrane receptors. The activated steroid receptors may sensitize the neuronal system for modulation by steroid hormones and regulate physiology and behavior. Such interactions could

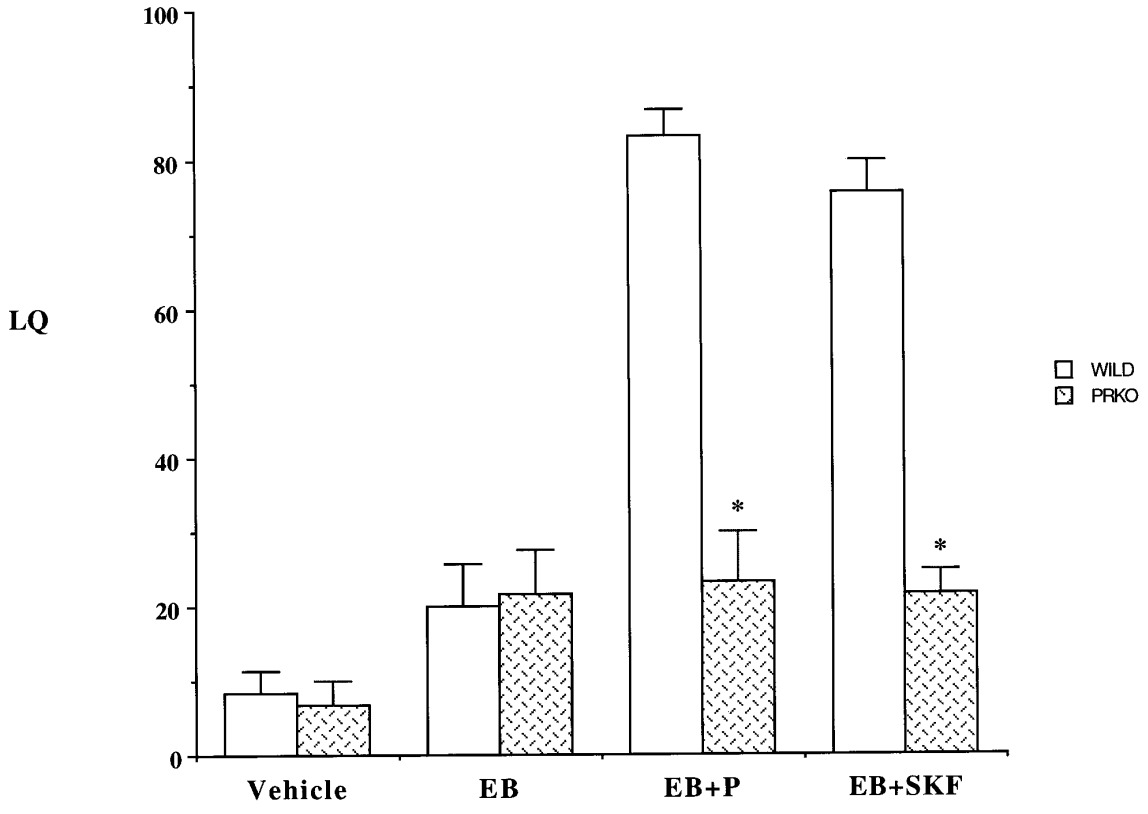

FIG. 4. Effect of icv administered dopamine D_1 agonist, SKF 38393 on lordosis response in PRKOs. Ovariectomized PRKOs and their wild-type littermates were primed weekly with EB + P and tested for 4 consecutive weeks. On Week 5, stainless steel cannulae were chronically implanted into the third cerebral ventricle. Following implantation of cannulae on Week 5, the mice were primed with EB on Week 6 and tested for sexual behavior 30 min following icv administration of D_1 agonist, SKF 38393 (50 ng). Control mice received vehicle (saline) or EB followed by saline or P 48 hr later. Statistically significant differences were seen in SKF- and P-facilitated responses of the PRKOs compared to the wild-type (*$P < 0.001$). $n = 6$ for each group. (Reproduced with permission from S. K. Mani, J. M. C. Allen, J. P. Lydon, B. M. Jericevic, J. D. Blaustein, F. J. DeMayo, O. M. Connely, and B. W. O'Malley. Dopamine requires the unoccupied progesterone receptor to induce sexual behavior in mice. *Mol. Endocrinol.* **10,** 1728–1737, 1996. © The Endocrine Society).

be a generalized mechanism by which neuronal responses to various hormonal and environmental signals are integrated in a coordinated and physiologically relevant manner.

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